Food Chemistry 230 (2017) 463–474

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Optimization of the recovery of high-value compounds from pitaya fruit by-products using microwave-assisted extraction

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ARTICLE INFO

Article history:
Received 2 May 2016
Received in revised form 8 March 2017
Accepted 11 March 2017
Available online 14 March 2017

Keywords:
Box-Behnken
HPLC–DAD–ESI/MS^n
Yellow pitaya
White-fleshed red pitaya
Fruit by-products
Microwave-assisted extraction

ABSTRACT

A green microwave-assisted extraction of high value-added compounds from exotic fruits' peels was optimized by Box-Behnken design using 3 factors: solid/solvent ratio, X_1, temperature, X_2, and extraction time, X_3. By using Derringer's desirability function, optimum extraction yields are obtained with $X_1 = 1/149.95$ g/mL, $X_2 = 72.27$ °C and $X_3 = 39.39$ min (white-fleshed red pitaya) and $X_1 = 1/148.96$ g/mL, $X_2 = 72.56$ °C and $X_3 = 5.02$ min (yellow pitaya) and a maximum betacyanin content is achieved with $X_1 = 1/150$ g/mL, $X_2 = 49.33$ °C and $X_3 = 5$ min. None of the factors influenced the extraction of phenolic compounds. Eighteen cinnamoyl derivatives, 17 flavonoid derivatives and 4 betacyanins were identified by HPLC–DAD–ESI/MS^n, 23 and 15 new compounds being described in yellow and white-fleshed red pitayas, respectively. These results indicate that it is possible to reuse these by-products to recover compounds for food and pharmaceutical industries.

1. Introduction

With approximately 1.3 billion tonnes of food and food by-products lost or wasted *per* year, agro-industrial wastes, such as fruit peels, become a potential source of high value-added functional compounds, which could be used in pharmaceutical, cosmetics and food industries (Gudiña et al., 2016; Kabir et al., 2015; Makris, Boskou, & Andrikopoulos, 2007; Matharu, de Melo, & Houghton, 2016). In an era of great environmental concern, food by-products valorisation and application of green extraction processes have gained much attention. Many innovative techniques, such as microwave-assisted extraction (MAE), accelerated solvent extraction (ASE), and supercritical fluid extraction (SFE), obey the principles of green extraction, such as the reduction of the energy employed and amount of solvent used for the extraction, being the greatest alternative for natural products' extraction. In addition, higher extraction yields are achieved most of the time (Armenta, Garrigues, & de la Guardia, 2015; Teo & Idris, 2014). Water seems to be the greenest solvent due to its non-toxicity, non-corrosiveness, non-flammability, environmentally benign nature, and abundance and availability at low cost. Thus, it seems the ideal solvent to extract polar compounds from natural sources.

There are several species belonging to the genus *Hylocereus*, popularly known as "pitaya", but only few of them are cultivated for fruit production. These exotic fruits can be distinguished by the colour of their peel and pulp. The commercialized *Hylocereus* species present red peel/white pulp [*Hylocereus undatus* (Haw.) Britton & Rose and *Hylocereus trigonus* (Haw.) Saff.], red peel/red pulp [*Hylocereus purpusii* (Weing.) Britton & Rose, *Hylocereus costaricensis* (Web.) Britton & Rose and *Hylocereus polyrhizus* (Web.) Britton & Rose] and yellow peel/white pulp [*Hylocereus megalanthus* (K. Schumann ex Vaupel) Ralf Bauer] (Le Bellec, Vaillant, & Imbert, 2006).

Besides human consumption, the fruits of *Hylocereus* species are very attractable raw materials for food industry for two main reasons: first, their peels account up to 33% of the fruit weight, and, in the case of *H. polyrhizus* peels, a pectin yield of 17% was obtained by Tang, Wong, and Woo (2011); secondly, peels and pulps are sources of natural colorants, namely betacyanins. Betacyanins are red- to purple-coloured betalains (absorbance from 530 to 545 nm) (Herbach, Stintzing, & Carle, 2006). These hydrophilic nitrogenous secondary metabolites are free radical scavengers (Suh et al., 2014), antilipidemic (Wroblewska, Juskiewicz, & Wiczkowski, 2011), anti-obesity and insulin resistant (Song, Chu,

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http://dx.doi.org/10.1016/j.foodchem.2017.03.061
0308-8146/

Xu, Xu, & Zheng, 2016) and antimicrobial (Canadanovic-Brunet et al., 2011) agents, among others. These compounds were already identified in several *Hylocereus* species by high performance liquid chromatography coupled to diode array detection (HPLC-DAD), HPLC coupled to tandem mass spectrometry (HPLC-DAD-MS^n), HPLC coupled to nuclear magnetic resonance (HPLC-NMR) and 2D NMR (Esquivel, Stintzing, & Carle, 2007; Kim et al., 2011; Stintzing, Conrad, Klaiber, Beifuss, & Carle, 2004; Stintzing, Schieber, & Carle, 2002; Tenore, Novellino, & Basile, 2012; Wybraniec & Mizrahi, 2002). In addition, polyphenols from *H. polyrhizus* displayed antioxidant (Esquivel et al., 2007; Kim et al., 2011; Ramli, Ismail, & Rahmat, 2014; Tenore et al., 2012), antiproliferative (Kim et al., 2011) and antimicrobial activities (Tenore et al., 2012). Total flavonoid and phenolic contents were only established for *H. undatus,* without a full chemical characterization (Kim et al., 2011) and no studies were previously performed with *H. megalanthus*. Thus, *Hylocereus* fruits are a rich source of antioxidant compounds, such as polyphenolic compounds and betacyanins, which may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated to oxidative stress. According to Scalbert, Manach, Moran, Rémesy, and Jiménez (2005) and to Khan (2016), the dietary intake of polyphenols has been estimated to ca. 1 g/day, flavonoids and phenolic acids corresponding to two-thirds and one-third of total daily phenolic intake, respectively. This amount is about 10 times higher than that recommended for the betacyanin betanin.

Response surface methodology (RSM) is a useful statistical tool to find the best set of independent variables or factors that produce the optimum response. In contrast to the traditional univariate procedures, in which each variable is studied separately, the multivariate systems provide more information about the variables and their interactions, all for a limited number of experiments (Candioti, de Zan, Cámara, & Goicoechea, 2014). Box-Behnken design (BBD), a type of RSM, is a second-order multivariate technique based on three-level incomplete factorial design. A three-factor BBD consists of three blocks of four experiments, where a two-factor variation is assayed with the third factor set at the centre point (Ferreira et al., 2007). BBD has been widely applied to optimize the extraction of bioactive compounds from natural sources (Grosso et al., 2014; Tsiaka et al., 2015).

The aim of this study was to find the best microwave-assisted extraction conditions to recover bioactive phenolic compounds and betacyanins from the peels of *H. undatus* and *H. megalanthus*. A BBD was applied to study the variation of three different extraction parameters: solid/solvent ratio, temperature, and time, using water as the extracting solvent. Moreover, to bridge the scarce information about the phenolic profile of both species and to improve the knowledge on betacyanin content, a complete characterization and quantification of these two classes of compounds was carried out, for the first time, by HPLC–DAD–ESI/MS^n and HPLC-DAD, respectively.

2. Materials and methods

2.1. Chemicals and standards

Acetonitrile and formic acid were bought from Merck (Darmstadt, Germany) and VWR (Fontenay-sous-Bois, France), respectively. 4-*O*-Caffeoylquinic acid was purchased from Chengdu Biopurify Phytochemicals Ltd. (Sichuan, China). 5-*O*-Caffeoylquinic acid, ferulic acid, isorhamnetin-3-*O*-rutinoside, kaempferol-3-*O*-rutinoside and isorhamnetin-3-*O*-glucoside were from Extrasynthèse (Genay, France) and *p*-coumaric acid, quercetin-3-*O*-rutinoside and a red beet extract diluted in dextrin containing betanidin-5-*O*-glucoside and its epimer isobetanidin-5-*O*-glucoside were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Plant material

White-fleshed red pitaya (lot no. 0465F21; from Vietnam) and yellow pitaya (lot no. 2745B21 and 0455F21, from Colombia) fruits were obtained from Henrique Fiel Lourenço, LDA (S. J. do Tojal, Portugal). Peels and pulps were separated and peels were lyophilized and powdered to a mean particle size below 910 µm.

2.3. Extraction

Aqueous extraction was performed in a MLS 1200 Mega high performance microwave digestion unit (Milestone, Sorisole, Italy) equipped with an HPR-1000/10 S rotor, at 600 W. Different combinations of solid/solvent ratio (1/50–1/150 g/mL), temperature (25–75 °C) and extraction time (5–65 min) were tested to optimize the extraction of phenolic compounds and betacyanins. The extracts obtained were centrifuged twice (2 × 10 min, 4000*g*) (Rotofix 32 A, Hettich Zentrigugen, Germany) to remove pectins and the supernatant was collected and freeze-dried.

2.4. Factorial design

In order to select the appropriate range for each factor, preliminary experiments were performed, where two factors were fixed and the third one was set at the lowest or at the highest level. A *t*-test was performed to analyse the significant differences at $p < 0.05$ (IBM SPSS Statistics 23). After setting the range of the factors, the software Design Expert (version 9, Stat-Ease Inc., Minneapolis, MN, USA) was used for experimental design, data analysis and model building.

Box-Behnken design (BBD) was applied to find out the optimum extraction conditions to obtain the highest extraction yield and content of phenolic compounds and betacyanins. Extraction yields (%) correspond to the ratio between the amount of dried extract obtained and the amount of peels used in the extraction, multiplied by 100.

The number of experiments (N) needed for the development of BBD is defined as:

$$N = 2k(k-1) + cp,$$

where k is the number of factors (or independent variables) and cp is the number of replicates at the centre point.

The independent variables (factors) chosen for this study, specifically the solid/solvent ratio (X_1), the temperature (X_2) and the extraction time (X_3) were combined in three different levels (−1, 0, 1) and coded according to the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X} \quad i = 1,2,3 \tag{1}$$

where x_i is the coded value of an independent variable, X_i is the actual value of an independent variable, X_0 is the actual value of an independent variable at the centre point, and ΔX is the step change value of an independent variable. The coded and uncoded levels of the three independent variables are given in Table S1. In total, 15 runs were performed in triplicate with three repetitions of the centre point (Table S1).

In order to predict the optimal responses (extraction yield, phenolic content and betacyanins' content), the following second-order polynomial equation was fitted to the experimental data:

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$